CXLI. THE CHEMICAL COMPOSITION OF TEETH.

III. THE VARIATIONS IN CHEMICAL COMPOSITION IN RELATION TO DENTAL STRUCTURE.

By JOANE HYLTON BOWES and MARGARET MARY MURRAY.

From the Department of Physiology, Bedford College, University of London.

(*Received April 8th, 1936.*)

Different theories exist as to the causes, remote and immediate, of dental caries, but it is probable that all theories admit of the view that the more perfect the structure of a given tooth, other things being equal, the less likely is that tooth to become carious. The relation between surface and histological structure of the teeth and the incidence of caries was investigated in children by Mellanby [1923; 1927, 2; 1934]. It was shown by her that when teeth were graded either in the hand or in the mouth for both the degree of hypoplasia and of caries, among the teeth which showed the more severe hypoplasia there was a greater incidence of caries. Hypoplastic teeth [Mellanby, 1927, 1] are rough and have pits and fissures, as opposed to perfect teeth which have a hard, smooth surface. It is easy to understand that the former would more easily harbour and favour the growth of the micro-organisms of caries; but the relationship between hypoplasia and caries might go farther than this and be in some way related to chemical composition or structure.

Thewlis [1934], as a result of X-ray analysis of human teeth, concluded that there were, judged from this particular physical aspect, three kinds of enamel, exhibiting variations in the amount of preferentially orientated apatite and in the degree of preferential orientation. Whether these classes of enamel can be related to the degree of imperfection of the surface structure, further work must decide. The aim of the present investigation was to see whether chemical analysis could establish any differences between perfect and imperfect (hypoplastic) non-carious teeth. The analyses were carried out on material obtained from the L.C.C. school dental clinics. All the extractions from 12 clinics were collected over a period of nearly 2 years. The total number of teeth obtained was about 10,000. The sound premolars (numbering over 300) were selected and carefully classified by examination with a lens and probe. Five classes were formed. The figures for the percentage occurrence of the teeth of the different classes are given.

			Occurrence %
(1)	Class N	No hypoplasia, or normal	4
(2)	Class Hy	Slight restricted hypoplasia	26
(3)	Class Hy +	More generalised hypoplasia	36
(4)	Class Hy + +	More severe and more generalised	23
(5)	Class Hy gross	Very severe, areas deficient of enamel	11

In classifying these teeth the method used was that of Mellanby [1927, 1]. She, however, graded into four classes and included a class Hy− which would correspond to Hy or come between N and Hy in the above classification.

It must be remembered that these figures do not apply to all teeth but to teeth of this one group, sound premolars, removed from children under 14 years of age for regulation purposes. The reason for the small percentage of non-hypoplastic (class N) teeth is probably that perfect teeth do not often occur in an overcrowded mouth. Teeth not fitting well into this classification, for example a few showing severe but very restricted hypoplasia, were discarded. Analyses of the separate enamel and dentine obtained as previously described [Bowes and Murray, 1935, 2] were made. Classes 2–4 inclusive were analysed quantitatively for ash, N, Ca, Mg, P, CO_2 and Cl by methods described in a previous communication [Bowes and Murray, 1935, 2], in which was set forth the chemical composition of the class Hy. In that communication references to methods and to previous investigations were given. So few perfect teeth were obtained that the separate analysis of class N had to be abandoned—(a few N teeth were included in the analyses of class Hy). Sodium and fluorine were not determined in all classes because of the relatively large amount of material required. Small variations in the amounts of these two elements are probably not significant in this particular group of analyses. Fluorine has not been included in the calculations because it is possibly not present in all teeth.

Mellanby [1929] noticed that on ashing in a crucible the teeth of dogs fed on different rachitogenic diets, the less well calcified teeth gave a black or grey ash, whereas the teeth of good structure gave a whiter ash. The weight of ash and the Ca content calculated as percentage of "wet" weight were less in the hypoplastic teeth of rachitic animals. The children from whom the teeth used for the analyses in the present investigations were obtained were not examined for evidence of past rickets, but since hypoplasia (gross) of the teeth is associated therewith [Wilson and Surie, 1930; Eliot *et al.*, 1933; Mackay and Rose, 1931], it might be presumed that the more severely hypoplastic teeth came from children who had had rickets. These human teeth, then, formed to some extent a series comparable with those of Mellanby's dogs. It was therefore of interest to repeat on these classified permanent teeth what might be called a rough test of hypoplasia. The teeth used for this purpose were all second maxillary premolars and of as nearly the same weight as possible. This was considered important because it was hoped in this way to minimise differences in modelling of the teeth and in the relative amount of enamel and dentine. The teeth were taken as soon after extraction as possible, having been kept moist, and were placed in saline in a refrigerator, if not used at once. They were brushed vigorously, the crowns cut off at the gingival line and the pulp cavities cleaned out. The crowns were dipped in alcohol and dried between filter-paper and put in an oven at 75° for a few minutes to remove the alcohol. They were weighed in a crucible, heated at first over a free flame and then with a blow-pipe till all smell of burning had gone; at this stage differences in the colour of the ash were noticed similar to those observed by Mellanby. Longer heating in a furnace, however, converted all samples into a white ash. Frequently this ash had a pinkish tinge. The final weights of the ashes were then taken and determinations of Ca made, the following results being obtained.

Class	Initial "wet" weight (g.)	Weight of ash (g.)	% Ash	Ca % in "wet" material
Hy	0·6698	0·5691	84·98	31·62
,,	0·6188	0·5229	84·50	32·64
,,	0·5628	0·4780	84·92	33·00
Hy +	0·6068	0·5084	83·79	30·42
,,	0·5801	0·4791	82·57	31·36
,,	0·6809	0·5590	82·10	30·96
Hy + +	0·6084	0·4942	81·23	29·73
,,	0·6612	0·5402	81·7	29·65
Hy gross	0·4700	0·3665	77·98	29·07
,,	0·5186	0·4127	79·58	29·71

The small but progressive decrease in the ash and Ca content from class to class represents a decrease in calcified material and indicates, either a relative decrease in the depth of the enamel, or a relatively greater organic content of the dentine, or a greater total area of poorly calcified areas and interglobular spaces in the dentine. It was found impossible to select teeth of the gross class of a weight equal to that of the others, no doubt because of the areas deficient in enamel; the enamel has a greater specific gravity than dentine. It would probably have been more satisfactory if slices of fresh dentine had been used in this test.

The composition of different enamels. The results of the detailed analyses of the enamels of the different classes are given in Table I.

Table I. *Analysis of enamels.*

% in dry enamel	Hy	Hy +	Hy + +	Hy gross	Apatite (carbonate)
Ash	95·38	95·20	94·76	94·67	—
N	0·156	0·161	0·153	0·208	—
Ca	37·07	36·85	36·29	35·81	—
P	17·22	18·01	18·04	17·72	—
CO_2	1·952	2·312	2·424	2·434	—
Mg	0·464	0·564	0·404	0·477	—
Cl	0·3	0·334	0·29	0·19	—
CO_2 % in ash	0·54	0·638	0·584	0·665	—
Ca : P : CO_2 (molar)	10 : 5·99 : 0·48	10 : 6·31 : 0·57	10 : 6·41 : 0·61	10 : 6·39 : 0·62	10 : 6 : 1
Ca/P	2·153	2·046	2·012	2·020	2·151
Ca/CO_2	18·99	15·94	14·97	14·71	9·10
P/CO_2	8·8	7·8	7·6	7·6	4·23

Determinations of Na and K were made in one class only. Hy had 0·25 % Na and a trace of K; the latter was not found spectroscopically.

Consideration of the figures makes it possible to draw several conclusions. Firstly the composition of the different enamels is very similar, which fact has in the past made many workers say that it is constant. But close consideration reveals differences. The loss in weight on ashing increases with increasing hypoplasia, giving a decreasing ash content. This could result from an increased content of combined CO_2 or H_2O, or organic matter. There is definitely an increased CO_2 content. The small decrease in Ca content with increase of hypoplasia is in keeping with the decrease in ash content. The phosphorus value does not vary in the same way but shows both a relative and an absolute increase with a consequent effect on the Ca/P ratio. For this reason it is to be emphasised that no analyses of the Ca content of tooth substance should be considered apart from the phosphorus content. The progressive decrease in Ca/P ratio and the consequent departure from the apatite value is considered by us to be one of the most significant points as regards the enamel constitution. The Mg values do not suggest that where the ratio is lower Mg has replaced Ca, for with the exception of the Hy+ class the Mg values show little variation. This would indicate that the increase in Mg content found in caries and pyorrhoea by various workers [Howe, 1926; Ulrich, 1925; Kaushansky, 1932] is a result and not a predisposing cause of the condition.

The amount of CO_2 in enamel does not permit the assumption that carbonato-apatite is the only compound present. The chlorine value decreases progressively from the sound to gross hypoplasia. The significance of this is hard to determine. Fränkel [1907] stated that with increasing age there was a decrease in halogen content but we are not dealing here with age differences. The highest chlorine value we have obtained was found in the enamel of sound molar teeth of old people, natives of India. All ashed samples of enamel have a small CO_2 content, the amount does not seem to vary much. The variations in the molar proportions are significant and show a progressive increase in phosphorus and CO_2.

In order to bring out points of difference between the different classes of teeth, calculations have been made to arrive at some idea of composition of these various enamels. Any calculations of composition are beset with difficulties because of lack of precise knowledge as to the manner in which the inorganic substance is laid down. It is generally accepted that various X-ray investigations have settled the fact that the chief constituent of bones and teeth is a form of apatite. But the existence in teeth of various cations and anions not present in calcium carbonate or hydroxy-apatite necessitates the consideration of the important point discussed fully by Robison [1932]. Robison's view is that, in the absence of more knowledge of the physicochemical system of calcifying tissues, it is dangerous to postulate the composition of the calcified substance. He prefers the view based on much experience that there is one bone and tooth compound, fundamentally a complex calcium carbonato-phosphate in which the Ca can be replaced by equivalent amounts of Mg, Na or K and in which the carbonate moiety can be replaced by $(OH)_2$ or Cl_2 and occasionally by minute traces of F_2. Such a replacement theory might explain the alterations in composition of bones with age and in fossil bone formation. In any case it must be admitted that from such a complex selection of ionic substances as exist in blood plasma, the possibility that the calcified substance will contain some of all the ions present is very great. Whether those other ions like Mg^{++} and Cl^- *etc.* are actually present combined in the bone compound or whether adsorbed or separately deposited has not been determined.

A detailed consideration of the results obtained on the enamel and dentine from the different grades of teeth has been made. One of the points considered was the calculation of the total cation (Ca^{++}, Mg^{++}, Na^+ and K^+) and anion ($PO_4^{\equiv}$, $CO_3^{=}$, Cl^-) concentrations. The following values were obtained (Table II).

Table II. *No. of cation and anion gram equivalents per 100 g. enamel.*

	Hy	Hy +	Hy + +	Hy gross
Cation	1·9032	1·901	1·8592	1·8413
Anion	1·7694	1·8564	1·8702	1·8374
Excess cation	0·1338	0·0446	– 0·0110	0·0039

There is then a progressive decrease of total cation and a less regular diminution of anion concentration. The better formed enamel is more basic.

If all the cations are calculated as equivalent Ca and compared with the P and CO_2 values, then only in the Hy grade can all the P be present as apatite. In no grade of enamel is all the apatite in the carbonato-form; some hydroxy-apatite must be present. We have calculated the possible composition of the different enamels, not so much to postulate the composition as to make comparisons between the different classes.

The Mg has been included in the apatite and where possible all the P has been placed as apatite. When an excess of Ca occurred it has been included as $CaCO_3$ and where an excess of P as $CaHPO_4$. The inclusion of the latter compound must be taken as provisional until a more satisfactory means of dealing with the excess P has been found.

This method of calculation gave the figures set out in Table III.

Table III.

	Hy	Hy +	Hy + +	Hy gross
Chloro-apatite $3Ca_3(PO_4)_2.CaCl_2$	4·397	4·91	4·251	2·768
Carbonato-apatites $3Ca_3(PO_4)_2.CaCO_3$ $3Ca_3(PO_4)_2.MgCO_3$	24·498	53·73	56·47	56·64
Hydroxy-apatite $3Ca_3(PO_4)_2.Ca(OH)_2$	64·51	33·81	26·47	27·99
Excess Ca as $CaCO_3$	2·028	Nil	Nil	Nil
Excess P as $CaHPO_4$	Nil	4·803	9·342	7·675

Such a table emphasises the effect which small differences in Ca and P have on the calculated composition. Facts which could be deduced from the anion and cation values are shown in this table. The diminishing total apatite, the increase of carbonato-apatite and the more acidic phosphorus compound as the hypoplasia increases are the notable points. Because of the different molecular weights of the different apatites it is better to compare the apatite calcium. If the value for the apatite P of Hy is put as 100, then the corresponding values for the other classes are: Hy+ 98, Hy+ + 92·5, Hy gross 92·5. The calculations then seem to bear out the deduction made earlier that enamel of the hypoplastic teeth contains less apatite.

Dentine. The analyses of the dentines never gave such consistent results as did those of the enamels. The inconsistencies encountered in the dentine are probably mainly due to the fact that even after eruption, and particularly soon after, new dentine is being laid down. This later and post-eruptive dentine deposited near the pulp, possibly because it is more slowly deposited, is often of better structure than the rest in a hypoplastic permanent tooth [Mellanby, 1927, 1]. In the collection of the dentine for analysis, some of this more highly calcified, as well as some of that near the enamel, which is often defective, was not included, so that though the dentine of the teeth of each class would be largely of good structure, nevertheless it can be seen that dentine can never be uniform throughout except in perfect teeth. The most characteristic differences between the dentine of teeth of good and bad structure is to be seen by histological investigation, showing the occurrence of interglobular spaces. But if there are differences in the composition of the calcified material, then our method should detect them. Determinations were carried out on dry and ashed dentine and the results given in Table IV were obtained. Sodium and potassium were not estimated in the separate classes. Class Hy+ dentine contained 0·19 % Na and 0·07 % K. These values were used in calculations.

Table IV. *Analysis of dentines (% in dry dentine).*

	Hy	Hy +	Hy + +	Hy gross	Apatite (carbonate)
Ash	71·09	70·64	70·17	70·28	—
N	3·43	3·247	3·371	3·449	—
Ca	27·79	27·54	27·27	26·96	—
P	13·81	14·35	13·61	13·5	—
CO_2	3·176	3·383	3·046	3·102	—
Mg	0·835	0·882	0·797	0·728	—
Cl	Nil	Nil	Nil	0·023	—
Ca : P : CO_2 (molar)	10 : 6·12 : 1·04	10 : 6·72 : 1·12	10 : 6·44 : 1·02	10 : 6·46 : 1·05	10 : 6 : 1
Ca/P dry	2·012	1·919	2·004	1·995	2·151
Ca/P ash	2·091	2·044	2·059	2·043	—
Ca/CO_2 dry	8·75	8·13	8·95	8·89	9·10
P/CO_2 dry	4·15	4·24	4·46	4·35	4·23

The results show less than the enamels in the way of progressive alteration of any one constituent. Slight decreases in ash, Ca, P and in the Ca/P ratio occur with increase of grade of hypoplasia. There was no increase in the Mg as the hypoplasia increased. This is worthy of note since an increase in Mg in whole crowns occurs in both caries and pyorrhoea [Ulrich, 1925; Kaushansky, 1932]. It is probable that the low Mg in gross hypoplasia is a general occurrence, for we have obtained several low figures for this value. The Ca/P ratios for all dentines, even after ashing, are less than that required by apatite. A comparison of the enamel and dentine values should be made, since many people consider them to be identical in all respects but organic content. The differences found were: (1) The Ca/P of ashed dentine (average) is 2·057, which is lower than the average enamel value of 2·140. (2) Dentine contains twice as much Mg as enamel. (3) Dentine has a higher CO_2 content, all of which is driven off by ashing. (4) The dentine substance, with one exception, does not contain combined chlorine. Dentine probably contains NaCl in interglobular spaces and lymph channels in its nourishing liquid, but this would have been washed out with water and all these Cl determinations, like those of sodium, were made after water extraction, in a dilute HNO_3 solution. The sodium content of dentine (not listed) was only 0·19 %.

The close correspondence of the dentine with carbonato-apatite seen in the molar proportions in Table III, is only apparent because no correction has been made for the Mg, which must make a considerable difference. Calculations of the probable composition of the dentines presented great difficulties. Having tried many methods we came to the conclusion that in dentine also apatite is the chief constituent and that more of this is in the carbonato-form. Other substances appear to be present, for instance $CaCO_3$ or $MgCO_3$ and an acidic phosphate compound. It was considered best to deal with anion and cation concentrations as a basis for comparison. These are set forth in Table V.

Table V. *No. of cation and anion gram equivalents per 100 g. of dentine.*

	Hy	Hy+	Hy++	Hy gross
Cation	1·4692	1·4606	1·4400	1·4188
Anion	1·4854	1·5468	1·4594	1·4521
Excess anion	0·0162	0·0862	0·0194	0·0333

Hence it is seen that whereas in enamel there is in most cases excess of positive equivalents, in dentine there is always an excess of negative equivalents. This fact and the Ca/P ratios point to the existence of some form of calcium phosphate other than tricalcium phosphate, a more acidic phosphate. Burns and Henderson [1935] came to a similar conclusion working with bone. Even if all the metals are expressed as the equivalent amount of Ca and the "corrected" Ca/P obtained, these ratios do not reach the apatite value. The corrected Ca/P ratios are: Hy 2·126, Hy+ 2·034, Hy++ 2·114, Hy gross 2·101. As to the comparison between the classes; the best class is the most basic and gives the highest Ca/P both actual and corrected. That is, the best dentine is a more basic dentine as the best enamel is also more basic.

A chemical theory of the causation of caries was put forward by Gassmann [1921]. He studied wisdom teeth and compared them with other teeth; his conclusion was that whereas in bones and teeth generally, the proportions of $Ca : P : CO_2$ were always 10 : 6 : 1, in wisdom teeth there was excess Ca and CO_2 over and above proportions. This excess Ca and CO_2 was in the proportion 4 : 1, which he took to mean that the formation of apatite was incomplete.

His theory was that the first formed compound in calcification was the co-ordination compound

$$Ca\left[\begin{matrix}OH\\OH\end{matrix}\!\!>\!Ca\right]_3.CO_3$$

called the "hexol salt", this by replacement of the hydrogen of the hydroxy-groups by $CaPO_3^-$ was converted into carbonato-apatite. The hexol salt was less stable, *i.e.* less resistant than apatite, so that when incomplete conversion took place, susceptibility to caries resulted. His argument was indirect, based on the known susceptibility of wisdom teeth to caries. The point of interest here is that the results put forward by the present authors indicate that hydroxy-apatite (as well as the carbonato-apatite) represents an abundant compound in the enamel of teeth. There is lack of agreement between us and Gassmann, in that his theory would correlate susceptibility to caries with a relative diminution of phosphorus, whereas we find invariably a relative increase of phosphorus with increase of hypoplasia.

In conclusion it would be well to emphasise that the results have shown that teeth do vary in their chemical composition but that these variations can only be seen in rather elaborate analyses of separated enamel and dentine. This investigation confirms by chemical evidence the X-ray findings that different kinds of enamel exist [Thewlis, 1934] and indicates that X-ray analysis of different classes of enamel should give significant results. The analyses are being extended to carious and pyorrhetic teeth.

Summary.

1. Analyses of separated enamel and dentine of teeth showing the different grades of hypoplasia defined in this paper were made. The determinations included ash, N, Ca, Mg, Na, K, P, CO_2 and F.

2. Small progressive variations in P content, Ca/P ratio and CO_2 content occurred.

3. With increase of hypoplasia the enamel showed definite alterations of composition, the most outstanding of which was the decrease of apatite content from 92 to 86 % and a corresponding increase of some other phosphorus compound.

4. The different dentines showed less regular variations in composition. Alterations in composition of the inorganic part, similar to those shown by the enamels, were found. The apatite decreased from 53 to 49 %.

5. Enamel and dentine differ in other respects than in the amount of organic substance, hence they should be analysed separately.

6. Calcium determinations alone are not sufficient to show up alterations of composition; they must be taken in conjunction with phosphorus and magnesium values.

We wish to record our thanks to the Medical Research Council for an expenses grant and for a personal grant to J. H. B. during the progress of this work.

We would also like to express our thanks and appreciation to the Sisters in charge of the L.C.C. dental clinics, for without their co-operation this work would have been impossible.

REFERENCES.

Bowes and Murray (1935, 1). *Biochem. J.* **29**, 102.
—— —— (1935, 2). *Biochem. J.* **29**, 2721.
Burns and Henderson (1935). *Biochem. J.* **29**, 2385.
Fränkel (1907). Deskriptive Biochemie. (Bergmann, Wiesbaden.)
Eliot, Souther, Anderson and Arnim (1933). *Amer. J. Diseases of Children*, **46**, 458.
Gassmann (1921). Beitrag zum Studium des chemischen Aufbaues des Knochen und Zahngewebes. (Bern.)
Howe (1926). *Dental Cosmos*, **61**, 1021.
Kaushansky (1932). *Dental Cosmos*, **74**, 568.
Mackay and Rose (1931). *Lancet*, **221**, 1230.
Mellanby, M. (1923). *Brit. Dental J.* **44**, 1.
—— (1927, 1). *Brit. Dental J.* **48**, 737.
—— (1927, 2). *Brit. Dental J.* **48**, 1481.
—— (1934). *Med. Res. Council Spec. Report*, No. **191**.
Robison (1932). Significance of phosphoric esters in metabolism. (New York Univ. Press.)
Thewlis (1934). *Brit. Dental J.* **57**, 455.
Ulrich (1925). *Dental Cosmos*, **67**, 1204.
Wilson and Surie (1930). *Ind. Med. Res.* **17**, 903.